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Survival and infectivity of *Heterorhabditis indica* Poinar in different formulations against pests of bitter gourd

P. S. GAYATHRI[1] and M. S. NISHA[2*]

[1]Department of Agricultural Entomology, [2]Department of Nematology
College of Agriculture, Vellayani, Kerala Agricultural University, Kerala, India

[*]**E-mail**: nisha.das@kau.in

ABSTRACT: Epilachna beetle (*Henosepilachna septima* Dieke) and melon fruit fly (*Zeugodacus cucurbitae* Coquillett) are serious pests of bitter gourd in Kerala. Studies were conducted to evaluate pathogenicity of Entomopathogenic nematodes (EPNs) against *H. septima and Z. cucurbitae* under laboratory conditions. NBAIR isolates of EPNs *viz. Heterorhabditis indica* Poinar and *Steinernema carpocapsae* Weiser were tested for their pathogenicity against 3rd instar larvae of *H. septima*. Among EPN species, *H. indica* @100 IJs recorded highest percentage mortality (100.00) followed by *S. carpocapsae* (90.00) at 48 hours after treatment (HAT). Three different formulations *viz.* sponge, talc and alginate gel of effective EPN strain was prepared and stored for 11 weeks. Infective juveniles stored in alginate gel formulation showed more than 50.00 per cent survival upto eight weeks after storage and recorded 72.22 per cent mortality of *H. septima* larvae at 72 HAT. Results of laboratory experiment on the effect of EPN formulation on adult emergence of fruit fly revealed that both alginate gel and talc based formulations of *H. indica* is equally effective to chemical. The study highlighted the efficacy of *H. indica* formulations as soil application for the management of *Z. cucurbitae*.

Keywords: Epilachna beetle, melon fruit fly, entomopathogenic nematode, shelf life, formulation

INTRODUCTION

Bitter gourd is cultivated throughout the year in Kerala and pests and diseases are major constraints. Among pests, *Henosepilachna septima* Dieke and *Zeugodacus cucurbitae* Coquillett are the major problems. Grubs of *H. septima* damage the crop by scraping the surface of leaves resulting in skeletonization while adults make semicircular holes on leaves causing great debilitation to the crop (Sreekala and Ushakumari, 1999). The most severe economic damage in cucurbits is caused by maggots of *Z. cucurbitae* by feeding inside the fruits and make them unfit for consumption. About 60-80 per cent yield loss has been reported in cucurbits due to pest attack (Shivalingaswamy *et al*., 2002). Even though the use of chemical pesticides is an easy approach to minimize pest population, it causes pesticide resistance and residues which results public health issues as well as considerable damage to natural ecosystem. As bitter gourd is an export-oriented crop, pest management using biopesticides is an important alternative to minimize use of chemical pesticides. EPNs of genus *Steinernema* and *Heterorhabditis* are lethal endo parasites of insects having a symbiotic relationship with bacteria of the genus *Xenorhabdus* and *Photorhabdus* respectively. The bacteria are carried in the gut of EPNs and injected into haemocoel of host causing septicaemia and death (Adams and Nguyen, 2002). Although the infective juveniles of EPNs can be stored in water under refrigerated condition, high cost and difficulties in maintaining quality reduce its acceptability as a component in IPM. So, development of formulations which can enhance its survival and infectivity lead to increased adoption of EPN technology for pest management. Hence the present study is undertaken to evaluate the pathogenicity and survival of EPN in different formulations and its effectiveness against *H. septima* and *Z. cucurbitae* in bitter gourd.

MATERIALS AND METHODS

Insect culture

H. septima, Z. cucurbitae and *Galleria mellonella* L. (greater wax moth) were maintained in the laboratory conditions. *H. septima* was reared using bitter gourd leaves in a rearing jar (30x20x20 cm). *Z. cucurbitae* was reared using ripened fruits of bitter gourd and last instar larvae were transferred to soil for pupation and emergence in pupation jar (30x20x20 cm). *G. mellonella* reared using artificial diet was used for mass multiplication of EPNs.

Table 1. Mortality percentage of *H. septima* at different concentrations of EPN

Treatments		Mortality* (%) Hours after treatment (HAT)					Emergence (IJs/insect)
EPN	Dose (IJs)	24	36	48	60	72	
H. indica	10	0.00 (0.00)^{b}	42.50 (40.61)^{c}	80.00 (63.80)^{bc}	100.00 (90.00)^{a}	100.00 (90.00)^{a}	$1.3x10^{4}$
	20	5.00 (9.21)^{b}	65.00 (53.84)^{b}	82.50 (65.46)^{b}	100.00 (90.00)^{a}	100.00 (90.00)^{a}	$2x10^{4}$
	50	5.00 (9.21)^{b}	80.00 (63.80)^{a}	95.00 (80.78)^{a}	100.00 (90.00)^{a}	100.00 (90.00)^{a}	$7.2x10^{4}$
	100	40.00 (39.16)^{a}	85.00 (67.50)^{a}	100.00 (90.00)^{a}	100.00 (90.00)^{a}	100.00 (90.00)^{a}	$9.8x10^{4}$
S. carpocapsae	10	0.00 (0.00)^{b}	12.50 (20.46)^{e}	50.00 (45.00)^{d}	94.87 (80.65)^{b}	100.00 (90.00)^{a}	$0.7x10^{4}$
	20	0.00 (0.00)^{b}	22.50 (28.22)^{d}	70.00 (57.16)^{c}	94.87 (80.65)^{b}	100.00 (90.00)^{a}	$1.3x10^{4}$
	50	0.00 (0.00)^{b}	47.50 (43.55)^{c}	80.00 (63.80)^{bc}	100.00 (90.00)^{a}	100.00 (90.00)^{a}	$3x10^{4}$
	100	2.50 (4.60)^{b}	50.00 (45.00)^{c}	90.00 (74.14)^{ab}	100.00 (90.00)^{a}	100.00 (90.00)^{a}	$9.1x10^{4}$
CD (0.05)		(9.440)	(6.983)	(11.677)	(7.362)	NS	

Figures in the parenthesis are arc sine transformed values *Mortality corrected using Abbott's formula

EPN culture

Two EPN strains, *S. carpocapsae* and *H. indica* (NBAIR isolate) were stored in tissue culture flasks at 15^{o}C in BOD incubator. Freshly harvested juveniles were collected through white trap method from infected *G. mellonella* cadavers and used for further experiments.

Pathogenicity of EPN against *H. septima*

Infectivity of *S. carpocapsae* and *H. indica* were assessed against 3rd instar grubs of *H. septima* at different concentrations (10, 20, 50 and 100 IJs/grub) under *in vitro*. Each treatment was replicated four times with ten grubs of *H. septima* in separate Petri plates. Mortality of grubs was noted at 24, 36, 48, 60 and 72 HAT. The infected cadavers were transferred to white trap and emerging IJs were collected and counted under stereoscopic microscope.

Preparation of sponge formulation

Polyurethane sponges were cut into rectangular pieces with dimension 3x3x2 cm. These sponges were

Table 2. Survival of infective juveniles of *H. indica* in different formulations

Treatment	Survival (%) at weekly intervals										
	1st week	2nd week	3rd week	4th week	5th week	6th week	7th week	8th week	9th week	10th week	11th week
Sponge	97.50 (83.62)^{a}	89.37 (71.02)^{b}	75.83 (60.62)^{c}	63.75 (52.99)^{c}	51.04 (45.59)^{c}	36.66 (37.24)^{c}	19.16 (25.88)^{c}	6.45 (14.60)^{c}	0.00 (0.00)^{c}	0.00 (0.00)^{b}	0.00 (0.00)^{b}
Talc	98.75 (86.77)^{a}	96.75 (81.04)^{a}	88.00 (70.03)^{b}	80.12 (63.58)^{b}	66.25 (54.51)^{b}	51.87 (46.07)^{b}	42.50 (40.68)^{b}	17.50 (24.44)^{b}	8.37 (16.56)^{b}	0.00 (0.00)^{b}	0.00 (0.00)^{b}
Alginate gel	100.00 (90.00)^{a}	98.75 (86.77)^{a}	94.60 (76.63)^{a}	88.20 (70.04)^{a}	82.65 (65.41)^{a}	75.00 (60.24)^{a}	66.75 (54.88)^{a}	61.00 (51.36)^{a}	32.50 (34.71)^{a}	26.25 (30.68)^{a}	19.40 (26.00)^{a}
Water	85.00 (68.02)^{b}	63.75 (53.01)^{c}	52.50 (46.44)^{d}	31.25 (33.93)^{d}	15.87 (23.29)^{d}	7.40 (15.63)^{d}	0.00 (0.00)^{d}	0.00 (0.00)^{d}	0.00 (0.00)^{c}	0.00 (0.00)^{b}	0.00 (0.00)^{b}
CD (0.05)	(9.718)	(7.333)	(5.2)	(4.063)	(3.997)	(5.25)	(4.181)	(4.538)	(3.656)	(3.426)	(2.739)

Figures in the parenthesis are arc sine transformed values

washed and air dried. The water holding capacity of sponge was determined by soaking in water and then squeezing the water out (Touray *et al*., 2020). Freshly harvested juveniles at a concentration of 1000 IJs/ml were added to each piece of sponge. These sponges were then packed individually in zip lock cover and stored at room temperature.

Preparation of talc formulation

Talc powder (50g) was added to 10 ml distilled water and 10 ml nematode suspension containing freshly harvested juveniles of 1000 IJs/ml was added to it (Jisna *et al*., 2019). The contents were mixed thoroughly till nematode suspension spread evenly on the talc and it was then stored at room temperature.

Preparation of alginate gel formulation

Freshly harvested juveniles were entrapped inside alginate beads using sodium alginate and calcium chloride complexing solution. 100 ml nematode suspension containing freshly harvested juveniles of 1000 IJs/ml was added to 2% sodium alginate solution and mixed it properly. Drops of this solution was added to 0.5 M calcium chloride dihydrate solution which was continuously stirred using a magnetic stirrer to form alginate beads (Kim *et al*., 2021). The formed beads were separated from the complexing solution after 20-30 minutes and then stored under room temperature.

Survival and virulence of infective juveniles in formulations

Different formulations of the effective EPN strain were prepared and stored at room temperature. Number of IJs surviving in sponge, talc, alginate gel and water were observed upto 11 weeks. Freshly harvested juveniles @ 1000 IJs/ml were used for making the formulations. Survival of IJs in sponge formulation was determined by soaking and squeezing each sponge in 50ml distilled water and number of IJs in 1ml of the suspension was counted. One gram of talc formulation was diluted in 5ml distilled water and then counted the number of IJs in 1ml. Alginate beads were diluted using 0.5M sodium citrate solution and IJs were counted. The virulence of IJs emerged from each formulation was tested against 3rd instar larvae of *H. septima* in comparison with freshly harvested juveniles.

Effect of EPN formulation against *Z. cucurbitae*

A laboratory experiment was conducted in Department of Agricultural Entomology, College of Agriculture, Vellayani to evaluate the efficacy of effective EPN formulations against the emergence of fruit fly adults

Table 3. Effect of *H. indica* in different formulations on the mortality of *H. septima* at 72 HAT

Treatments	*Mortality (%)										
	1st week	2nd week	3rd week	4th week	5th week	6th week	7th week	8th week	9th week	10th week	11th week
Sponge	100.00 (90.00)^{a}	100.00 (90.00)^{a}	91.42 (75.18)^{bc}	89.74 (71.32)^{cd}	86.48 (68.67)^{b}	68.57 (55.97)^{c}	60.00 (50.80)^{c}	33.33 (35.11)^{c}	0.00 (0.00)^{d}	0.00 (0.00)^{c}	0.00 (0.00)^{c}
Talc	100.00 (90.00)^{a}	100.00 (90.00)^{a}	94.28 (80.12)^{ab}	92.30 (75.99)^{bc}	89.18 (70.80)^{b}	85.71 (68.04)^{b}	71.42 (57.79)^{b}	66.66 (54.88)^{b}	37.83 (37.93)^{c}	0.00 (0.00)^{c}	0.00 (0.00)^{c}
Alginate	100.00 (90.00)^{a}	100.00 (90.00)^{a}	100.00 (90.00)^{a}	97.43 (85.33)^{ab}	91.89 (75.60)^{b}	85.71 (68.04)^{b}	77.14 (61.82)^{b}	72.22 (53.30)^{b}	43.24 (41.09)^{b}	41.02 (39.73)^{b}	22.22 (28.12)^{b}
Water	100.00 (90.00)^{a}	94.59 (80.40)^{a}	85.71 (68.04)^{c}	76.92 (61.75)^{d}	62.16 (52.08)^{c}	42.85 (40.83)^{d}	0.00 (0.00)^{d}	0.00 (0.00)^{d}	0.00 (0.00)^{d}	0.00 (0.00)^{c}	0 (0.00)^{c}
Freshly harvested juveniles	100.00 (90.00)^{a}	100.00 (90.00)^{a}	100.00 (90.00)^{a}	100.00 (90.00)^{a}	100.00 (90.00)^{a}	100.00 (90.00)^{a}	100.00 (90.00)^{a}	100.00 (90.00)^{a}	100.00 (90.00)^{a}	100.00 (90.00)^{a}	100.00 (90.00)^{a}
CD (0.05)	NS	NS	(10.595)	(10.117)	(7.503)	(6.094)	(5.873)	(6.009)	(3.013)	(3.923)	(0.00)

Figures in the parenthesis are arc sine transformed values

*Mortality corrected using Abbott's formula

from soil in comparison with recommended biocontrol agent (*Beauveria bassiana* NBAIR Bb5 @ 20g/L), botanical (neemazal 1% @ 0.2% + tween 80 @ 1%) and standard insecticide (chlorantraniliprole 18.5SC@ 0.3 ml/L). *Z. cucurbitae* infested fruits were collected from local farmers and the full grown larvae hopped from infested bitter gourds were transferred to a rearing cage. Clean fresh fruits were regularly introduced to the cage to enhance oviposition and larval development. Last instar larvae of length 7 to 11mm were collected from infested fruits in the cage for experiment.

The experiment was laid out in Completely Randomized Design (CRD) with seven treatments and three replications. Two effective formulations of selected EPN strain were prepared at a concentration of one lakh IJs/ml. The experiment was done in plastic containers filled with soil up to a height of 5 cm and the treatments were given to it. Then, ten late instar larvae of melon fruit fly were inoculated into each replication of the treatment along with a piece of bitter gourd fruit. Observations on the emergence of fruit flies from each treatment were taken at 10 and 15 DAT.

Statistical analysis

The tabulated data were subjected to statistical analysis using one-way analysis of variance after suitable transformations. The analysis was done using General R-shiny based Analysis Platform Empowered by Statistics (GRAPES) software (Gopinath *et al*., 2020).

RESULTS AND DISCUSSION

Pathogenicity of EPN against *H. septima*

In the study, *H. indica* and *S. carpocapsae* were evaluated for their pathogenic effect on 3rd instar larvae of *H. septima* in Petri plate bioassay (Table 1). *H. indica* @ 20, 50 and 100 IJs/larva recorded 5.00, 5.00 and 40.00 per cent mortality at 24 h after exposure. But no mortality of *H. septima* was observed in *H. indica* @ 10 IJs/ larva and *S. carpocapsae* @ 10, 20, and 50 IJs/larva. After 36 h of exposure, *H. indica* @ 100 IJs/larva recorded 85.00 per cent mortality and that of *S. carpocapsae* recorded 50.00 per cent mortality of *H. septima*. At 48 h after exposure, 100.00 per cent mortality was recorded with 100 IJs of *H. indica*. Increased susceptibility of *H. septima* to EPN in this study is in conformity with that of Abdel-Moniem and Gesraha (2001) who conducted a laboratory experiment on the pathogenicity of *Heterorhabditis taysearae* Shamseldean, *Heterorhabditis bacteriophora* Poinar and *S. carpocapsae* on different instars of *Epilachna chrysomelina* Fabricius and reported that *Heterorhabditis* sp. caused highest mortality of 2nd instar larvae of *E. chrysomelina* with mortality of 30.00 and 71.40 per cent at two and four days after treatment respectively. The superiority of *H. indica* over *S. carpocapsae* on the mortality of *H. sepima* in this study is attributed to the presence of apical hook in *Heterorhabditis* spp. which can rupture insect body wall more easily compared to *Steinernema* spp. where they lack the apical hook (Lewis *et al.*, 2006). Both *H. indica* and *S. carpocapsae* showed cent per cent mortality of *H. septima* in all concentrations (10, 20, 50 and 100 IJs) at 72h after exposure.

In the study, it was observed that the mortality percentage of *H. septima* by *H. indica* @ 20 and 50 IJs increased from 5.00 to 100.00 on increased exposure time from 24 to 72 h. Increased time of exposure from 36 to 72 h increased the mortality percentage of *H. septima* from 12.50 to 100.00, 22.50 to 100.00, 47.50 to 100.00 and 50.00 to 100.00 by *S. carpocapsae* @ 10, 20, 50 and 100 IJs respectively. This is in line with the findings of Gupta *et al.* (2008) who observed that the mortality of 3rd instar larvae of *Spodoptera litura* Fabricius increased (22.50 to 100.00 per cent) with exposure time (24 to 96 h) when treated with *S. carpocapsae* @ 80 IJs/ larva.

It was observed that mortality percentage of *H. septima* increased from 42.50 to 85.00 and 80.00 to 100.00 percentage at 36 and 48 h after exposure respectively, when concentration of IJs of *H. indica* increased from 10 to 100 IJs/larva. The relationship of mortality on time of exposure and concentration was substantiated by the findings of Adiroubane *et al*. (2010) who observed that increase in dosage decreased the time of exposure of *Steinernema siamkayai* Stock against *S. litura, Plutealla xylostella* Linnaeus, *Leucinodes orbonalis* Guenee, *Earias vitella* Fabricius and *Cnaphalocrocis medinalis* Guenee.

Cadavers infected with *H. indica* were reddish brown in colour and that of *S. carpocapsae* were dark brown. Emergence of IJs was highest for *H. indica* @ 100 IJs with an emergence of $9.8x10^4$ and it was followed by *S. carpocapsae* @ 100 IJs with $9.1x10^4$. *H. indica* @ 10, 20 and 50 IJs recorded an emergence of $1.3x10^4$, $2x10^4$ and $7.2x10^4$ respectively while *S. carpocapsae* @ 10, 20 and 50 IJs recorded an emergence of $0.7x10^4$, $1.3x10^4$ and $3x10^4$ respectively.

Survival and virulence of IJs in different formulations

Based upon the pathogenicity test, *H. indica* was selected as the effective EPN strain against *H. septima*. Thus, *H. indica* was formulated into four different formulations *viz.*, alginate gel, talc, sponge and water for the evaluation of survival and virulence of IJs stored

Table 4. Effect of *H. indica* on the emergence of fruit fly

Treatment	Emergence (%)*		Percentage reduction over control*	
	10 DAT	15 DAT	10 DAT	15 DAT
Alginate gel-based formulation of *H. indica* @ 4g/L+ tween 80 (1%)	3.33 (6.145)^{ab}	16.66 (23.36)^{a}	90.00	83.34
Talc-based formulation of *H. indica* @ 20 g/L+ tween 80 (1%)	3.33 (6.145)^{ab}	16.66 (23.85)^{a}	90.00	83.34
Beauveria bassiana NBAIR Bb5 @20 g/L	10.00 (15.00)^{b}	53.33 (46.92)^{b}	69.99	46.67
Neemazal 1% @ 0.2% + tween 80 (1%)	0.00 (0.00)^{a}	96.66 (83.85)^{cd}	100.00	3.34
Chlorantraniliprole 18.5SC@ 0.3 ml/L	0.00 (0.00)^{a}	13.33 (21.14)^{a}	100.00	86.67
Tween 80 (1%)	0.00 (0.00)^{a}	90.00 (75.00)^{c}	100.00	10
Untreated	33.33 (35.21)^{c}	100.00 (90.00)^{d}	-	-
CD (0.05)	(13.628)	(14.192)	-	-

*Figures in the parenthesis are arc sine transformed values

in these formulations. In the study, highest survival percentage of *H. indica* IJs (100.00) was observed in alginate and it was followed by talc (98.75) after one week of storage (Table 2). More than 50.00 per cent survival was obtained in alginate, talc and sponge until 8th, 6th and 5th week of storage respectively at room temperature. It is substantiated by Grewal (2002) that the viability of IJs decrease as the energy reserves got depleted over time. IJs of *H. indica* survived well in alginate and talc formulation than sponge and water in the present study. In case of alginate gel formulation, IJs are entrapped inside a liquid core where nematode movements are restricted to an extent. This also reduces the metabolic activity of IJs. Moreover, IJs in alginate gel are protected from UV radiation, desiccation and temperature (Grewal 2000). In sponge and water, IJs are free to move which reduces its lipid energy reserves and this attributes to the low survival percentage of *H. indica* in those formulations. While in talc formulation, IJs are subjected to anhydrobiotic condition which induces quiescence and thus reduced the metabolic rate of IJs (Kim *et al*., 2021). This contributes to the higher survival rate of IJs in talc compared to sponge and water. A study conducted by Jisna *et al.* (2019) on the survival of *Oscheius rugaoensis* in different formulations (talc, saw dust, alginate gel, water dispersible granules and compost-charcoal powder mixture) reported 80.60 and 68.00 per cent survival of *O. rugaoensis* in alginate gel and talc respectively in the 6th week of storage at 30^{o}C. Touray *et al.* (2020) reported that the survival percentage of *H. bacteriophora* IJs stored in different sponge types at 27^{o}C ranged from 89.30 to 93.70 during the first week of storage. Nagachandrabose (2022) studied the survival of *H. bacteriophora* (KKMH1) in alginate gel, talc and sponge wherein he reported more than 50.00 per cent survival of IJs up to 10th, 8th and 7th week in alginate gel, talc and sponge respectively at 25^{o}C. Likewise, Lalitha *et al*. (2022) also conducted an experiment on the survival of *S. carpocapsae* in arabic gum and alginate beads where they reported 93.30 per cent survival of IJs after 6 weeks of storage.

IJs stored in alginate, talc and sponge formulations for four weeks recorded 12.50 to 17.50, 60.00 to 67.50,

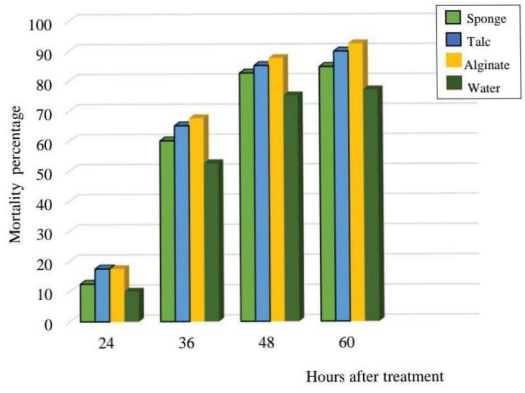

Fig. 1 Infectivity of *H. indica* stored for 4 weeks in different formulations against *H. septima* at 24, 36, 48 and 60h after exposure

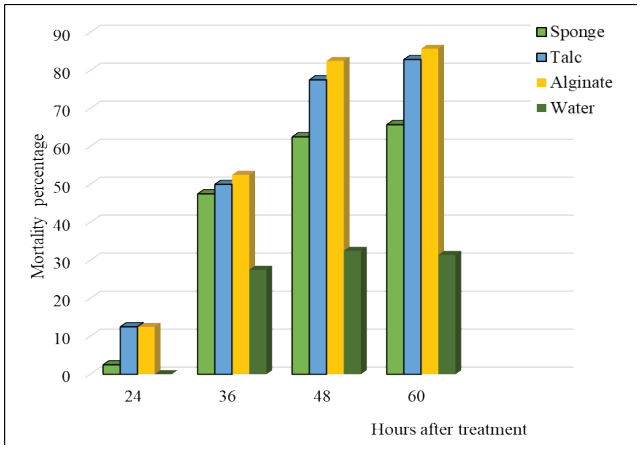

Fig. 2. Infectivity of *H. indica* stored for 6 weeks in different formulations against *H. septima* at 24, 36, 48 and 60h after exposure

82.50 to 87.50, 84.61 to 92.30 and 89.74 to 97.43 per cent mortality of 3rd instar larvae of *H. septima* at 24, 36, 48, 60 and 72h after exposure respectively (Fig. 1). After 6 weeks of storage, IJs stored in alginate, talc and sponge formulations recorded 2.50 to 12.50, 47.50 to 52.50, 62.50 to 82.50, 65.71 to 85.71 and 68.57 to 85.71 per cent mortality of *H. septima* at 24, 36, 48, 60 and 72 HAT respectively. At the same time, IJs stored in water recorded 0.00, 27.50, 32.50, 31.42 and 42.85 per cent mortality of *H. septima* at 24, 36, 48, 60 and 72 HAT (Fig. 2). More than 50.00 percent mortality of *H. septima* by *H. indica* juveniles stored in alginate gel and talc was observed upto 8 weeks (Table 3). Similar study was conducted by Nagachandrabose (2022) where he reported more than 50.00 percent mortality of *E. vitella* by *H. bacteriophora* (KKMH1) upto 9 weeks of storage in alginate gel and 7 weeks in talc. Results of the study confirmed virulence of IJs stored in alginate and talc formulation against *H. septima*.

Effect of EPN formulation against *Z. cucurbitae*

Efficacy of *H. indica* in formulations was tested against last instar larvae of melon fruit fly (*Z. cucurbitae*) by evaluating its effect on the emergence of adults from treated soil. Alginate gel-based formulation of *H. indica* @ 4g/L + tween 80 (1%) and talc-based formulation of *H. indica* @ 20 g/L + tween 80 (1%) were used for the experiment. The effect of these formulations was compared with biocontrol agent, *B. bassiana* NBAIR Bb5 20 g/ L, botanical, neemazal 1% @ 0.2% + tween 80 (1%) and chemical, chlorantraniliprole 18.5SC@ 0.3 ml/L.

The results showed that both alginate gel-based formulation of *H. indica* @ 4g/L+ tween 80 (1%) and talc-based formulation of *H. indica* @ 20 g/L + tween 80 (1%) showed significant reduction in the emergence percentage of *Z. cucurbitae* over untreated control. The effect of alginate gel-based formulation of *H. indica* @ 4g/L + tween 80 (1%) and talc-based formulation of *H. indica* @ 20 g/L + tween 80 (1%) recorded a reduction in emergence of 83.34 per cent over untreated control after 15 days of treatment (Table 4). At the same time, chemical recorded a reduction percentage of 86.67 which was similar to that of EPN formulations. This clearly indicates that the application of EPN in soil reduced adult emergence and its effect was similar to that of chemical treatment. The pathogenicity of EPN against *B. cucurbitae* was earlier studied by Sheela *et al.* (2002) where they reported 100.00 per cent mortality of *B. cucurbitae* larvae treated with *Rhabditis* sp. @ 200IJs larva^{-1} after 72 hours of exposure. Usman *et al.* (2021) conducted a potted soil assay on the pathogenicity of EPNs against *Bactrocera zonata* Saunders and *Bactrocera dorsalis* Hendel and reported that *H. bacteriophora* recorded 95.74 and 86.88 per cent mortality in larvae and 71.27 and 67.65 per cent mortality in pupae of *B. zonata* and *B. dorsalis* respectively. In the present study, neemazal 1% @ 0.2% + tween 80 (1%) recorded 96.66 per cent emergence at 15 DAT while there was no emergence for the treatment at 10 DAT. It is in agreement with the findings of Khan *et al.* (2007) who reported that the commercial neem formulation (Nimbicidine) did not show any effect on the pupation of *B. curcurbitae* and *B. dorsalis* while it delayed the pupation by 2-3 days from control. The effect of EPN was superior than *B. bassiana* NBAIR Bb5 20 g/L which showed 53.33 per cent emergence of *B. cucurbitae* at 15 DAT. It is in contrast with the study conducted by Amala (2010) where she reported 98.29 per cent mortality of *B. cucurbitae* with *B. bassiana* @

$2x10^7$ spore/ml. The variation in results might be due to difference in concentration levels. The emergence of adults increased after 15 days in all the treatments and only 33% emergence was observed in control 10 days after treatment. This may be due to the variation in pupal period of melon fruit fly from 7 to 13 days (Dhillon *et al.*, 2005). This study reveals the biocontrol potential of *H. indica* against the emergence of *Z. cucurbitae* adults from soil. This strategy can be effectively used for the management of fruit flies in integrated pest management programme of bitter gourd.

The study highlighted the biocontrol potential of *H. indica* against *H. septima* and *Z. cucurbitae*. It also revealed that *H. indica* can be stored in alginate gel and talc formulations for the better shelf life of IJs. An effort needs to be directed towards developing more viable and cost-effective formulations for the commercial success of EPN as a biocontrol agent.

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